JOURNAL OF ANIMAL SCIENCE
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J Anim Sci 1996. 74:2622-2632.

Operant Analysis of Chronic Locoweed Intoxication in Sheep[1]

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ABSTRACT: Five sheep were fed a 10% locoweed (*Oxytropis sericea*) pellet or alfalfa pellets for 3- to 5-wk periods to determine the effects of intermittent locoweed ingestion on operant responding; three controls were fed alfalfa pellets for 22 wk. Sheep were trained to respond to a multiple schedule with a fixed ratio (FR) 5 and fixed interval (FI) 50 s as major elements; performance was reinforced with rolled barley. Locoweed-treated sheep decreased ($P <$ .05) FR response rate after 4 wk of locoweed feeding, but this decrease first appeared during the first recovery period (wk 6). The FR response rate of intoxicated sheep did not return to baseline during the remainder of the study and differed from controls during most of the study. Controls did not deviate ($P >$ .05) from their FR baseline except during wk 2. Sheep did not stabilize on the FI component. As locoweed-treated sheep became progressively more intoxicated, they altered their pattern of FR responses, with longer post-reinforcement pauses, and a slower overall FR rate. Intoxicated sheep ingested an average of .21 mg swainsonine$\cdot$kg$^{-1}\cdot$d^{-1}. Overt signs of intoxication were noted when two sheep were stressed on wk 17. These two sheep had neuroviseral vacuolation typical of locoweed poisoning, whereas the three remaining locoweed-treated sheep that were euthanatized 5 wk later showed little histologic evidence of intoxication. Our findings indicate that "on-off" or cyclic grazing of locoweed ranges should be approached cautiously. Such a grazing program may be feasible because of the rapid resolution of histologic pathology; however, an initial toxic insult of 4 wk seems to be excessive, even at low doses, because sheep may exhibit persistent behavioral abnormalities that require >6 wk to resolve.

Key Words: Sheep, Operant Responding, Poisonous Plants, Loco Weed, *Oxytropis*, Swainsonine

J. Anim. Sci. 1996. 74:2622–2632

Introduction

Locoweed (*Astragalus* and *Oxytropis* spp.) intoxication is a major cause of livestock losses on rangelands throughout the world (James and Panter, 1989). As implied by the common name, ingestion of locoweeds can cause maniacal behavior in the latter stages of intoxication, as well as more subtle alterations in behavior such as nervousness, loss of proprioception, ataxia, and anorexia (James, 1972).

The locoweed toxin is the indolizidine alkaloid swainsonine (Molyneux and James, 1982). Swainsonine inhibits several intracellular mannosidases, leading to accumulation of oligosaccharides in lysosomes, abnormal glycoprotein synthesis, and eventual cell death (Dorling et al., 1989; Elbein, 1989). In the central nervous system, this is seen as vacuolar degeneration of both neurons and glia, which in chronic intoxication may result in swelling of axon hillocks and branching of dendrites (Walkley and Siegel, 1989; Stegelmeier et al., 1994). Swainsonine is rapidly metabolized and excreted (Stegelmeier et al., 1995a). Lesions appear within days when feeding begins (Van Kampen and James, 1969, 1970), and when locoweed ingestion ceases, the cytoplasmic vacuoles disappear rapidly (Stegelmeier, unpublished data). Even so, there may be permanent residual damage in nervous tissue of animals that have crossed a threshold of intoxication (Walkley and Siegel, 1989).

The first objective of this study was to determine whether operant procedures (Ferster and Skinner, 1957) could be used to assess the degree of intoxication in sheep fed locoweed. A second objective,

[1]We thank Tracy Weber and Kermit Price for invaluable assistance in the operant laboratory, Phil Kechele and Jane Chambers for tissue preparation, and the animal caretakers of the Poisonous Plant Lab. for animal feeding and care. We also gratefully acknowledge the cooperation of Clive Arave in all aspects of the operant analysis.

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Received December 8, 1995.
Accepted June 20, 1996.

assuming that the first objective was successful, was to determine the impacts of cyclic (i.e., on and off) locoweed consumption on sheep responses in an operant setting. It may be possible to reduce the risk of toxicosis on locoweed-infested pastures by recurrently grazing at high stock densities, such that individual animals periodically ingest only moderate amounts of locoweed, and are then allowed a recovery period. The second objective was investigated by experimentation in a controlled operant environment.

Materials and Methods

Operant Procedures. Twelve crossbred whitefaced female sheep with an initial weight of 77 ± 9 kg were used. Subjects were very tractable, having been handled several times per week in the previous year. Subjects were habituated over a period of several weeks to the two .6- × 1.5-m operant chambers, then trained to respond in the chambers. Because chambers were not soundproof, a white noise generator (7,500 Hz) provided background noise. Subjects were trained to break a beam of infrared light focused across a 14-cm circular plexiglass key with their noses; a keylight that varied in color and intensity was situated behind the beam as a discriminative stimulus. The key was backlighted with either a red or a white bulb. Subjects received feed from a feed bucket delivered to the subject via a pneumatic ram (Cate et al., 1978), which was controlled either manually or by computer. Initial behavior was shaped by manually providing access to rolled barley in the feed magazine as responses gradually came closer to breaking the light beam (i.e., successive approximations). Eventually, trained sheep were given 3-s access to grain as reinforcement for successful completion of each schedule component. Each response generated a 1,000-Hz tone.

All components of the operant schedule were essentially rules that determined whether the subjects were reinforced (i.e., presented grain) at that moment. For this schedule, we used three different components or rules: a fixed interval (**FI**), a fixed ratio (**FR**), and differential reinforcement of other behavior (**DRO**). During the FI component, the presentation of a reinforcer depended on the subject's behavior and the passage of time; the rule for reinforcement during the FI was that the first response after 50 s had passed was reinforced. During the FR component, the presentation of a reinforcer depended only on the subject's behavior; the rule was that a reinforcer was provided after every five responses (**FR5**). During the DRO segment, reinforce-

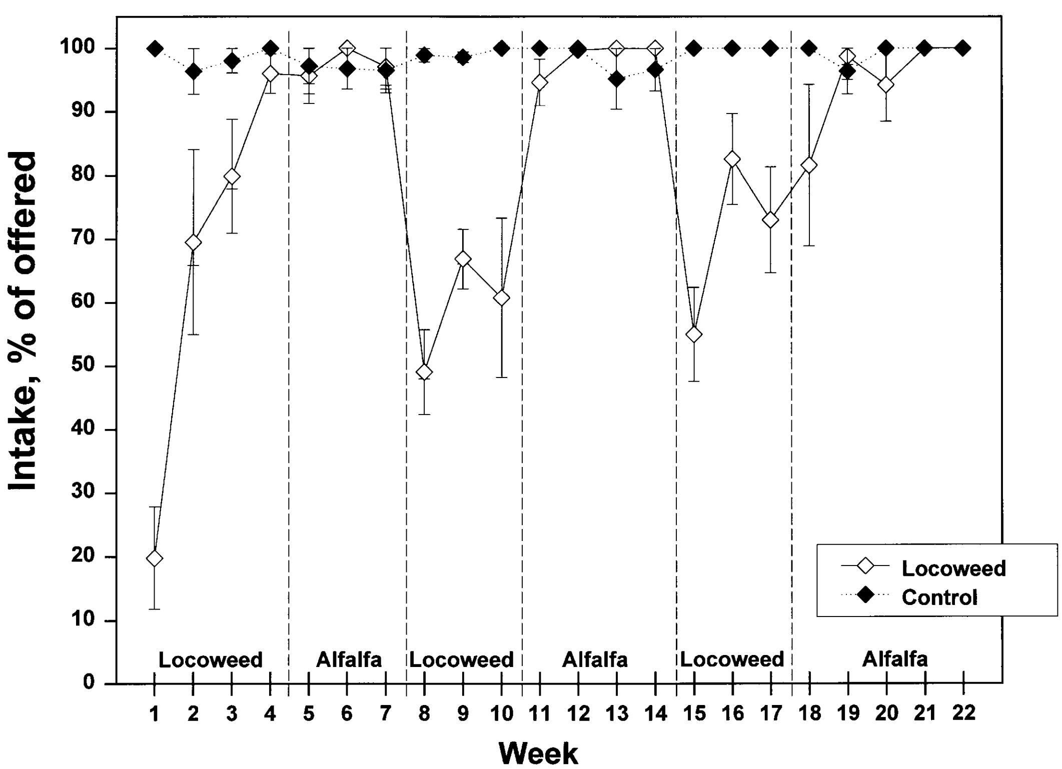


Figure 1. Mean pellet intake (% of offered ± SE) by sheep offered either 10% locoweed pellets or a 100% alfalfa hay pellet during the 22-wk study. Sheep on the locoweed treatment were given locoweed pellets during periods marked as locoweed, and they were fed alfalfa pellets during periods marked as alfalfa. Individual sheep were offered pellets at 1.6% of body weight.

ment was given if and only if no responses were made during a 10-s period. Any response during this period reset the clock for another 10 s, whereas absence of responding was rewarded with the delivery of a reinforcer.

Subjects were trained initially on an FR schedule that gradually increased from FR1 to FR5. When sheep performed this task (i.e., FR5) adequately, a 10-s DRO contingency was introduced. Finally, a 50-s FI was added to the schedule. The final multiple schedule included the FR 5 component (keylight white), which subjects completed four times, a single 10-s DRO (keylight off), a FI50 (keylight red), then a single 10-s DRO (keylight off). Trained subjects typically repeated this schedule sequence a number of times (i.e., loops) during a session.

The 28-V keylights differed in intensity from the white (5.5 watt) bulb to the red (1.7 watt) bulb. Although it has not been unequivocally established that sheep have color vision, they do have rods and cones (Munkenbeck, 1982), and our subjects were able to discriminate the different colors and(or) intensities of light (i.e., responses were controlled by the schedule).

Our rationale for this multiple schedule was based on previous behavioral toxicological studies (Seiden and Dykstra, 1977; Laties, 1982; Laties and Wood, 1986). The FR and FI schedules differ greatly in sensitivity to many drugs and environmental toxicants (Seiden and Dykstra, 1977; Laties, 1982; Laties and Wood, 1986). Ratio schedules are based on the number of responses, whereas interval schedules are based on time. Reductions in FR response rate lead to reduced reinforcement, whereas reductions in FI response rate may not produce any change in the number of reinforcers delivered (Laties and Wood, 1986). The FR-FI multiple schedule has been recommended as a general screening technique in behavioral toxicology (NAS, 1977). We included the DRO component to further evaluate the effects of locoweed intoxication on time-based responding. Because intoxicated sheep are often described as being "nervous" or "excited," we felt that subjects might respond inappropriately during this schedule component as the toxicosis progressed.

Eight sheep eventually stabilized on the multiple schedule; two sheep were discarded, and another pair were used as companion animals. Sheep are very gregarious, and isolation in the operant chambers induced erratic responses. We alleviated this nervousness by providing a companion that could not interfere with the subject, but was in constant visual and auditory contact through an adjacent wire mesh barrier.

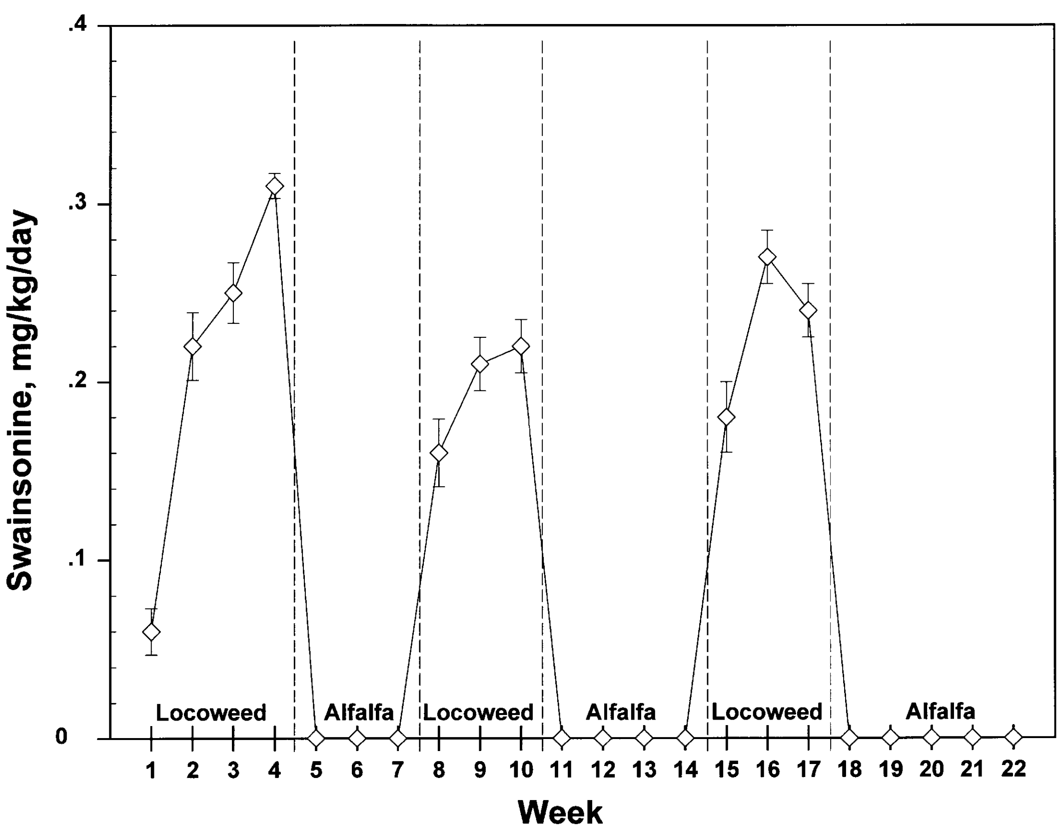


Figure 2. Mean swainsonine intake ($mg \cdot kg^{-1} \cdot d^{-1} \pm SE$) for locoweed-treated sheep during the various weeks of the study. The 10% locoweed pellet contained 20 mg swainsonine/kg (DMB).

Two subjects were run simultaneously in operant chambers, each with companion animals; chambers were 7-m apart in the same large room. We used the MED-PC software system with MEDSTATE notation for real-time control of multiple operant chambers (Tatham and Zurn, 1989). Cumulative records were recorded on an IBM-compatible computer using the MED Soft Cumulative Recorder (MED Associates, Georgia, Vermont). Operant sessions were 12 min in length. During training, sheep were run every weekday. As responses became stable, we still ran sheep each weekday, but used data only from sessions on Tuesday, Wednesday, and Thursday of each week. After training was completed, baseline for each sheep was established using the Tuesday through Thursday performance for analysis. Three consecutive weeks of stable responses were required to establish baseline before treatment. Criteria used for stability were as follows: FR rate ± .75 responses/min; total reinforcers ± 3/session; total loops ± .5/session; FR or FI DRO errors ≤ 2. A typical normal subject had an FR response rate of about 12 responses/min, 45 reinforcers/session, 7 loops/session, and only rarely made a DRO error.

Locoweed Collection and Feeding. Astragalus mollissimus (woolly loco) was collected fresh during June 1982 in Union County, NM, air-dried at 25°C, and ground to pass a 2-mm screen. The material was stored at room temperature in plastic bags until use. Swainsonine is stable indefinitely at room temperature. In 1991 the swainsonine concentration was determined by extracting plant material with hot methanol, followed by gas chromatographic analysis (Molyneux et al., 1989).

Locoweed pellets were made commercially by mixing 10% ground locoweed with 90% (wt/wt) ground alfalfa hay (*Medicago sativa*). Sheep were maintained initially on a 100% alfalfa pellet diet and fed individually at 1.6% of body weight, a level slightly above maintenance considering grain consumed during operant sessions (NRC, 1985). After the operant baseline was established, five randomly selected sheep were offered 10% locoweed pellets at 1.6% of body weight during three periods. The first locoweed feeding period was 4 wk and was followed by feeding of alfalfa pellets for a 3-wk recovery period. During the second cycle, treated sheep received locoweed during a 3-wk period, followed by 4 wk of alfalfa feeding; the final cycle included 3 wk of locoweed feeding followed

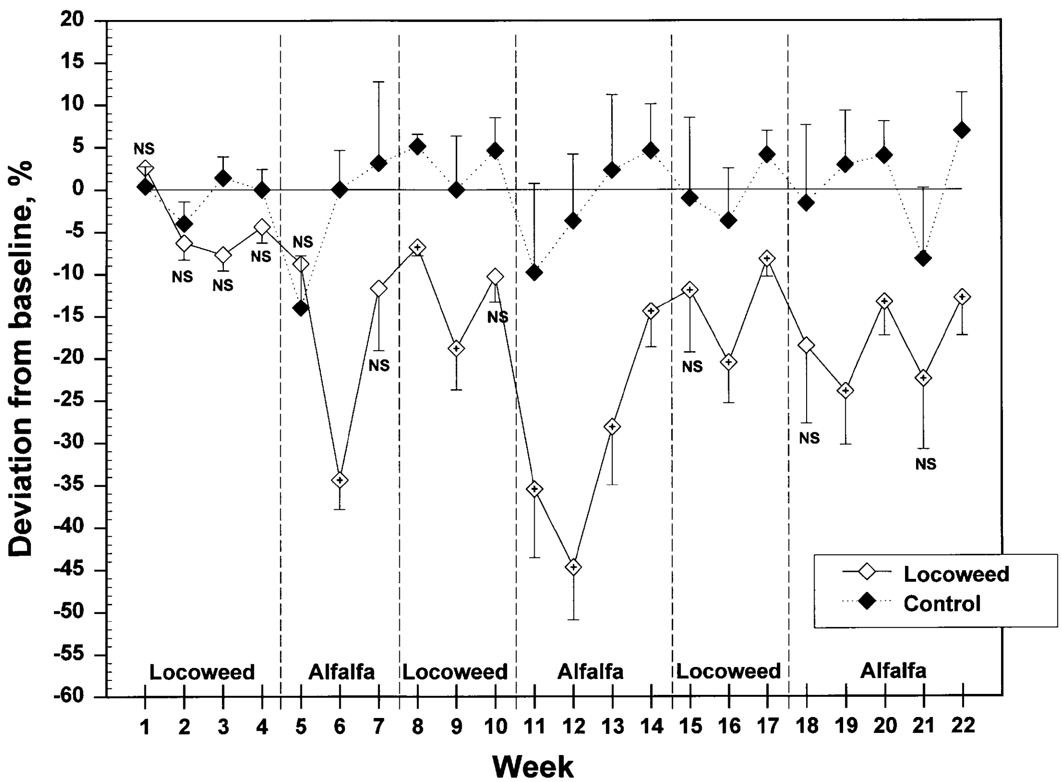


Figure 3. Mean deviation from baseline (% ± SE) for the total number of reinforcers obtained by sheep in 12-min operant sessions. Sheep in the locoweed treatment were fed locoweed pellets during periods marked as locoweed and alfalfa hay pellets during periods marked as alfalfa; control received only alfalfa hay pellets. Baseline was established for each individual sheep during a 3-wk period before treatments began. Each data point is an average of three sessions (i.e., Tuesday, Wednesday, and Thursday). The + in the symbol indicates that the data point differs from 0 ($P \leq .05$); controls did not differ from 0 in any week. The NS by a data point indicates that the mean for the locoweed treatment did not differ ($P > .05$) from controls.

by a 5-wk recovery period. Three control sheep received only alfalfa pellets during the study. Grain consumption during operant sessions was also recorded.

Histopathology. At the end of locoweed feeding on wk 17, two locoweed-treated sheep were humanely killed and necropsied; tissues were collected for histologic evaluation. Five weeks later, the remaining sheep were killed and necropsied. Tissues were prepared for histopathological examination using standard procedures. All protocols and procedures were conducted under veterinary supervision with the approval of the Utah State University Institutional Animal Care and Use Committee.

Data and Statistical Analysis. Feed consumption (pellets: percentage of amount offered; grain: kilograms eaten) was analyzed using a general linear model procedure (SAS, 1987). The model included treatment (locoweed or controls), with sheep nested within treatments, and repeated measurements over 22 wk. Sheep were considered a random factor. Significant week × treatment interactions were examined using *t*-tests.

In a two-pronged approach for operant variables, the locoweed treatment was compared with controls, and additionally both treated and control groups were compared with their respective baselines (Perone, 1991). Daily operant responses and associated variables were averaged to obtain weekly means. The number of reinforcers, FR response rate, and number of loops were analyzed as the percentage of deviation from baseline. The number of FR and FI DRO errors (i.e., responses during subsequent DRO) were analyzed without manipulation. The repeated measures model included treatment, animals nested within treatment, week, and the week × treatment interaction. Additionally, for the number of reinforcers and loops, and FR response rate (all expressed as percentage of deviation from baseline), a *t*-test was used to test (α = .05) the null hypothesis H_o: $\bar{x} = 0$ vs the two-tailed alternative H_A: $\bar{x} \neq 0$ for each group's weekly mean. All statistical analyses were performed using SAS (1987).

Results

Feed and Swainsonine Intake and Concentration

Sheep offered locoweed pellets ate less (P = .05) compared to control consumption of alfalfa pellets

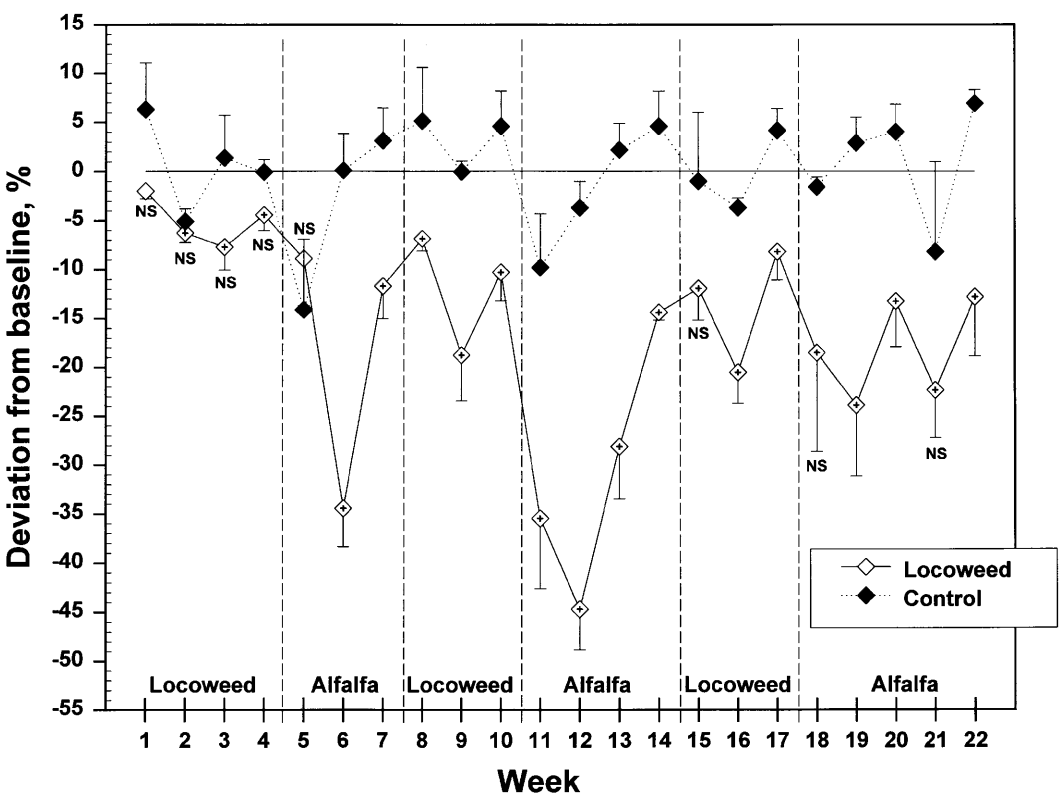


Figure 4. Mean deviation from baseline (% ± SE) in the fixed ratio (FR) response rate of sheep in 12-min operant sessions. Sheep in the locoweed treatment were fed locoweed pellets during periods marked as locoweed and alfalfa hay pellets during periods marked as alfalfa; controls received only alfalfa hay pellets. Baseline was established for each individual sheep during a 3-wk period before treatments began. Each data point is an average of three sessions (i.e., Tuesday, Wednesday, and Thursday). The + in the symbol indicates that the data point differs from 0 ($P \leq .05$); controls did not differ from 0 in any week except for wk 2. The NS by a data point indicates that the mean for the locoweed treatment did not differ (P > .05) from controls.

(Figure 1). There were differences among weeks ($P < .05$), and the treatment × time interaction was significant ($P < .05$). Each week that locoweed pellets were reintroduced to the locoweed group, pellet intake decreased ($P < .05$) compared to controls, then pellet intake rebounded somewhat during subsequent weeks (Figure 1). Grain intake during operant sessions did not differ ($P > .05$) between the treatment groups; there were week effects ($P < .001$), but no treatment × time interaction ($P > .05$). Grain consumption averaged .46 and .63 kg/session for the locoweed and control groups, respectively.

Pellets contained .002% swainsonine (**DMB**). Swainsonine intake averaged .21 mg/kg BW/d for locoweed-treated sheep for the periods when locoweed was fed (Figure 2). Treated sheep ingested a daily average of .21, .19, and .22 mg swainsonine/kg BW during the three locoweed feeding periods, respectively.

Operant Variables

Total Reinforcers Obtained. During the first locoweed feeding period (4 wk), there were no weekly treatment effects on the number of reinforcers the sheep obtained (Figure 3), nor did treatments deviate significantly ($P > .1$) from baseline. When the locoweed group was shifted to alfalfa pellets during wk 5 to 7, locoweed-treated sheep obtained fewer reinforcers compared to their baseline during wk 6 ($P < .01$), and treated sheep also differed in the number of reinforcers obtained ($P < .01$) from controls. Locoweed-treated sheep recovered during wk 7 but deviated ($P < .01$) from baseline and differed from controls ($P < .05$) during wk 8 and 9 when locoweed feeding resumed. When the second alfalfa feeding period commenced during wk 11, the locoweed treatment deviated from baseline ($P < .01$) and differed from controls ($P < .05$) and continued to deviate from baseline during wk 12 to 17. During wk 11 to 17, the locoweed treatment also differed from controls ($P < .05$), except during wk 15. During recovery in wk 18 to 22 (i.e., alfalfa feeding), the locoweed treatment deviated from baseline during all weeks except for wk 18. The locoweed treatment also differed ($P < .05$) from controls during wk 19, 20, and 22.

Fixed Ratio Rate. The treatment and control groups differed ($P < .01$) from baseline during wk 2; however,

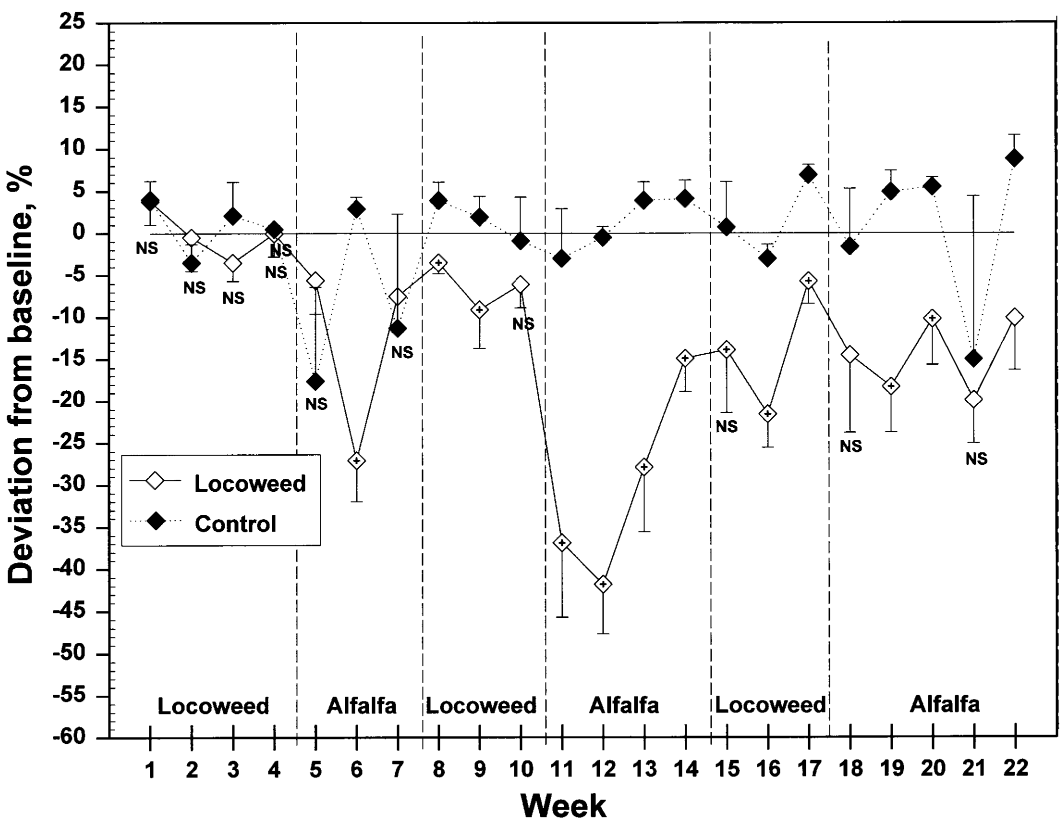
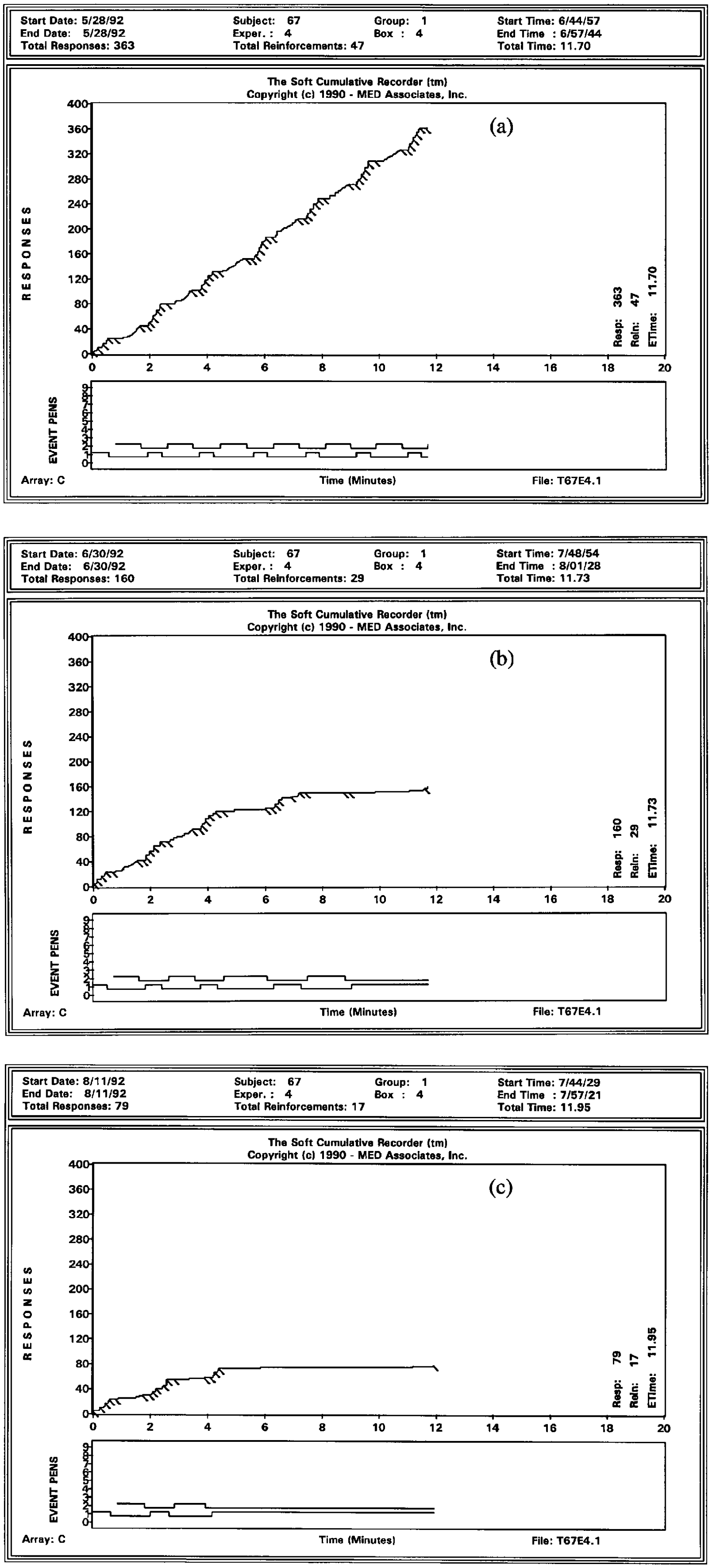


Figure 5. Mean deviation from baseline (% ± SE) in the number of loops (i.e., number of times through the multiple schedule) by sheep in 12-min operant sessions. Sheep in the locoweed treatment were fed locoweed pellets during periods marked as locoweed and alfalfa hay pellets during periods marked as alfalfa; controls received only alfalfa hay pellets. Baseline was established for each individual during a 3-wk period before treatments began. Each data point is an average of three sessions (i.e., Tuesday, Wednesday, and Thursday). The + in the symbol indicates that the data point differs from 0 ($P \leq .05$); controls did not differ from 0 in any week. The NS by a data point indicates that the mean for the locoweed treatment did not differ ($P > .05$) from controls.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End Date: 5/28/92
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Exper. : 4
Total Reinforcements: 47
Group: 1
Box : 4
Start Time: 6/44/57
End Time : 6/57/44
Total Time: 11.70
The Soft Cumulative Recorder (tm)
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(a)
RESPONSES
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Rein: 47
ETime: 11.70
EVENT PENS
Array: C
Time (Minutes)
File: T67E4.1
Start Date: 6/30/92
End Date: 6/30/92
Total Responses: 160
Subject: 67
Exper. : 4
Total Reinforcements: 29
Group: 1
Box : 4
Start Time: 7/48/54
End Time : 8/01/28
Total Time: 11.73
The Soft Cumulative Recorder (tm)
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(b)
RESPONSES
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Rein: 29
ETime: 11.73
EVENT PENS
Array: C
Time (Minutes)
File: T67E4.1
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End Date: 8/11/92
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Subject: 67
Exper. : 4
Total Reinforcements: 17
Group: 1
Box : 4
Start Time: 7/44/29
End Time : 7/57/21
Total Time: 11.95
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controls came to normal levels of responding during wk 3 and 4, whereas the locoweed-treated sheep had a reduced FR rate during wk 3 and 4 that deviated ($P < .03$) from baseline (Figure 4). For unknown reasons, the mean FR rate during wk 5 for both treated sheep and controls was reduced ($P > .1$) and variability was high. Locoweed-treated sheep showed a significant deviation from baseline during wk 6 to 22; they differed ($P < .05$) from controls in all remaining weeks, except for wk 15, 18, and 21 (Figure 4). Controls did not differ ($P > .1$) from baseline at any time except during wk 2.

Number of Loops. Locoweed feeding had no effect on the number of loops (i.e., number of times through the multiple schedule) until treated sheep were in the first recovery period during wk 6, when treated sheep deviated from baseline ($P < .01$) and differed from controls ($P < .01$; Figure 5). After wk 8, treated sheep showed significant deviations from baseline, except for wk 10, 18, 21, and 22. Treated sheep differed from controls in the number of completed loops in all weeks after wk 8, except for wk 10, 15, 18, and 21 (Figure 5).

DRO Errors. In general, sheep made few or no DRO errors. Neither the number of FR nor of FI DRO errors (i.e., responses during DRO) was affected by locoweed feeding. Treated sheep did not deviate from baseline ($P > .1$), nor did they differ from controls ($P > .1$) for this variable.

Pattern of Responding. The locoweed treatment altered the response pattern of treated sheep over time. Cumulative response records for a locoweed-treated subject on three dates are shown in Figures 6; a control sheep is shown in Figure 7. The slash (/) represents the presentation of the reinforcement, and the slope of the line between reinforcers indicates the rate at which the sheep was responding. Steeper slopes represent higher rates of responding. Presentation of reinforcements is often clustered in groups of seven, as a subject would obtain four reinforcers for completing the FR 5 portion (4x), then be presented with the DRO, and obtain one reinforcer at the conclusion of the 10-s DRO. This would initiate the 50-s FI, with the subject again obtaining a reinforcer after the first response at the end of the interval, then obtaining the seventh reinforcer at the end of the 10-s DRO. Locoweed intoxication increased the duration of the post-reinforcement pauses (Figure 6). Additionally, intoxicated sheep had slower FR response rates. Slower response rates led to intoxicated sheep obtaining fewer reinforcers than did controls (Figure 7) during a typical 12-min session. Although we did not examine the FI responses in detail due to instability, on occasion a locoweed-treated subject would have an extended period of nonresponding during the FI. It was more typical, however, for treated subjects to pause or quit during an FR segment.

Pathology. The two sheep euthanatized at wk 17 were depressed and lethargic. When stressed they often trembled and struggled against restraints. No significant gross lesions were identified in these sheep; however, there was moderate but distinct vacuolation of neurons (Figure 8), thyroid follicular epithelium, renal tubular epithelium, pancreas glandular epithelium, and tissue macrophages in the spleen and lymph nodes. The treated and control sheep that were euthanatized at wk 22 were clinically normal and the vacuolar lesions suggestive of locoweed intoxication had apparently resolved, assuming these lesions were present at wk 17. The locoweed-treated sheep necropsied at wk 22 did have small numbers of dystrophic axons or “spheroids” in the white tracts of the cerebellum and medulla.

Discussion

Locoweed intoxication had a generally depressing effect on operant responding. Intoxicated sheep had longer post-reinforcement pauses (as shown by the cumulative records) and slower FR response rates compared to controls. Some response variables were more sensitive to intoxication (e.g., FR rate) than were others (e.g., total reinforcers). The number of DRO errors was completely insensitive to degree of intoxication.

Figure 6. A typical cumulative record for sheep #1 from the locoweed treatment working under a multiple schedule, explained below, on three dates. These individual records correspond to wk (a) 3, (b) 8, and (c) 14 in the 22-wk study. The slash (\) represents the presentation of the reinforcement, and the slope of the line between reinforcers indicates the rate at which the animal was responding. Steeper slopes represent higher rates of responding. Presentation of reinforcement is often clustered in groups of seven, as a subject would obtain four reinforcers for completing the fixed ratio (FR) 5 portion (4x), then be presented with the differential reinforcement of other behavior (DRO), and obtain one reinforcer at the conclusion of the 10-s DRO. This would initiate the 50-s fixed interval (FI), with the subject again obtaining a reinforcer after the first response at the end of the interval, then obtaining the seventh reinforcer at the end of the 10-s DRO. Event pen #1 is for the FR component and event pen #2 is for the FI component; when the event pen is in the “up” position, this portion of the schedule is in effect. When both event pens are in the “down” position, either the DRO is in effect, or the subject is being reinforced for successful completion of the DRO (i.e., no responses made).

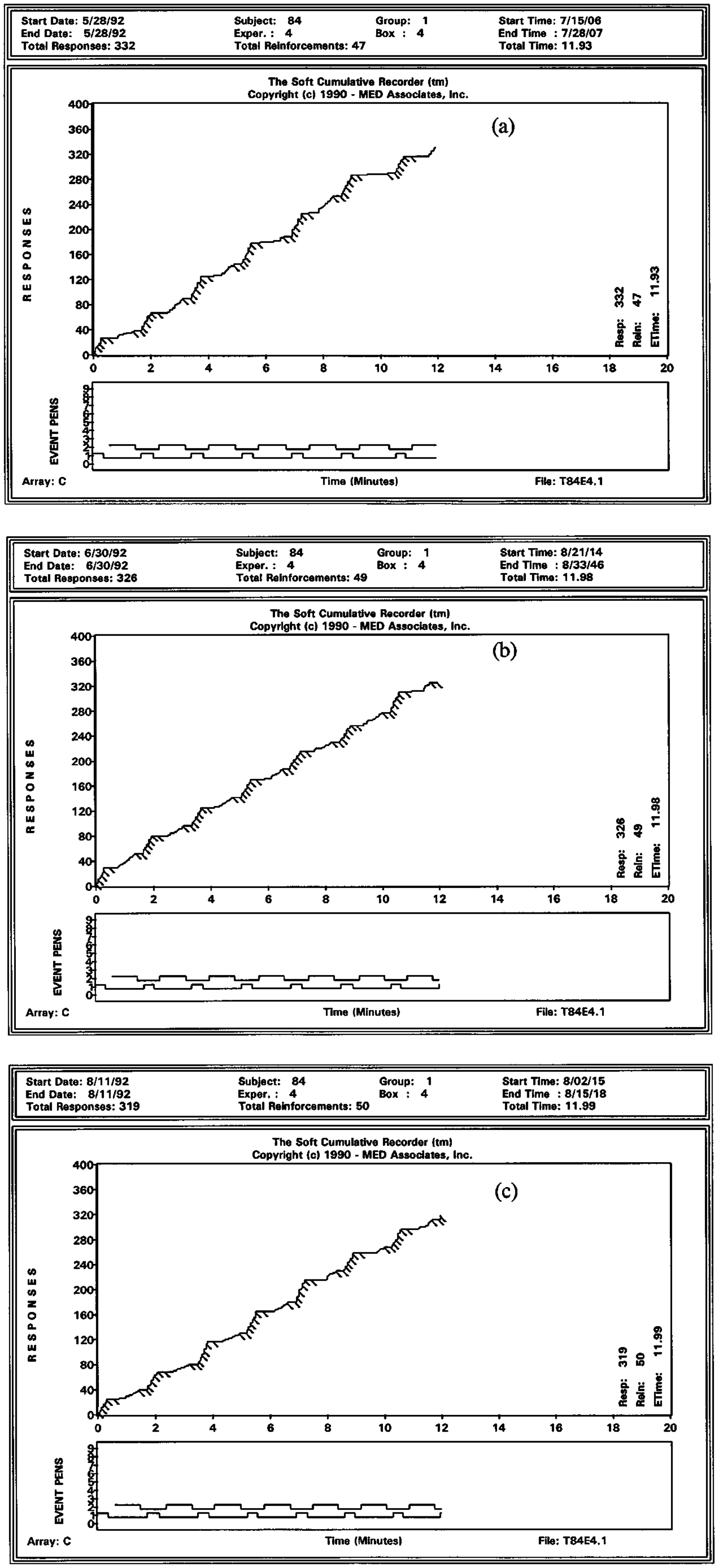

Figure 7. A typical cumulative record for sheep #6 from the controls working under a multiple schedule on three dates. These individual records correspond to wk (a) 3, (b) 8, and (c) 14 in the 22-wk study. For an explanation, see legend for Figure 6.

During the first locoweed-alfalfa feeding cycle (wk 1 to 7), locoweed sheep showed a severe decrease in responding during wk 6, even though alfalfa feeding began in wk 5, indicating a time lag between feeding the toxin and the behavioral impacts of intoxication. This lag effect has been noted in other studies on the pathology of locoweed intoxication (B. Stegelmeier, unpublished data). Frequently the clinical signs of intoxication are most severe several days after locoweed exposure. Although the etiology of this delay is unknown, it may result from decreased neuronal function as poisoned cells begin to recover, or as a result of increased inflammation as damaged cells and cellular debris are cleaned up and metabolized.

The locoweed pellet contained a relatively low concentration of swainsonine, and intake was sometimes low because the pellets were not well accepted at times. Swainsonine intake during the first locoweed feeding period averaged .21 mg/kg, suggesting that the threshold dose for mild intoxication in sheep is lower than the .30 mg/kg dose found for rats (Stegelmeier et al., 1995b). Further controlled studies must be conducted to establish the minimum toxic dose of locoweed in livestock.

The reduced FR rate during wk 6 to 22 suggests that locoweed-treated sheep did not recover from the initial 4-wk toxic insult, even at the relatively low levels fed in this study. These sheep did generally recover clinically and histologically after wk 17. It may be that our operant analysis was more sensitive and the continued decrease in FR responding was due to residual sequelae of intoxication. It is also possible that the sheep were not monitored long enough for them to return to baseline response rates. These findings suggest that caution must be used in any “on-off” or cyclic grazing scheme, because low-dose intoxication does cause operant and pathologic changes that seem to require many weeks or perhaps months to resolve. Additional studies are needed to better document the dose and duration of intoxication required to produce disease and to better define the permanent sequelae of poisoning.

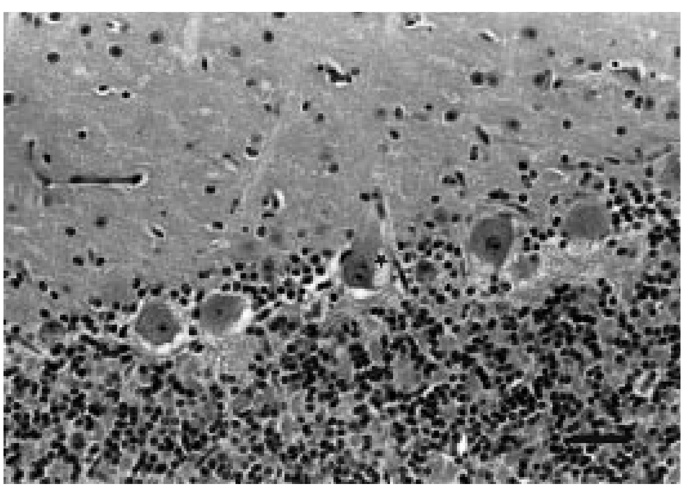

Figure 8. Photomicrograph of the cerebellum of a sheep intoxicated by locoweed and euthanatized on wk 17. Notice the vacuolated Purkinje cells (star). Bar=100 μm.

When two of the locoweed-treated sheep were euthanatized at the end of wk 17, they were stressed by transporting and holding them in a trailer. These stressed sheep showed typical signs and histologic pathology of intoxication. We assume that these lesions were present in the remaining sheep at wk 17. The neurological lesions seemed to resolve over the next 5 wk; the sheep that were dosed with locoweed and later necropsied at wk 22 did not have typical vacuolar lesions of poisoning. These sheep did have residual lesions (axonal degeneration and spheroids) and they never completely returned to normal operant responding, again suggesting that there are residual sequelae of locoweed poisoning.

Erratic operant results were noted during the last recovery period (wk 18 to 22). This can be partially explained by the removal from the study of two intoxicated sheep at the end of wk 17. Of the three remaining locoweed-treated sheep, we judged that two were more severely intoxicated than the third based on clinical signs; the smaller sample size and increased variability decreased the sensitivity of the analysis.

Sheep achieved stability in terms of the FR response rate, total number of reinforcers gained during a session, number of errors committed during the DRO segment, and the number of loops completed during a session. Training sheep to perform on this multiple schedule took several months of intensive effort, and some sheep did not stabilize on any schedule component and were removed from the study. Performance during the FI component of the schedule was the least stable. Typically, trained subjects (i.e., rats, pigeons) make few responses early in the interval then increase the rate of responding as the end of the interval approaches (i.e., FI scallop). Our sheep sometimes responded in this way, but at times even control subjects would respond excessively during the interval (Figure 7). Van Gelder et al. (1973) used two operant procedures to examine lead intoxication in sheep, and they found that an FI schedule was not sensitive to the toxic insult.

The locoweed pellet was a novel food and was not well-accepted by the sheep. Each time locoweed was offered anew, the locoweed sheep decreased intake for one to several weeks. After the initial feeding period, locoweed consumption never did return again to baseline, and it may be that sheep made some association between the locoweed feed and the gradual onset of the locoweed disease. This was apparently not an anoretic response (Prichard et al., 1990), because late in the study, when offered alfalfa pellets, locoweed-treated sheep consumed virtually all of the offered food. This feed intake pattern may have influenced animal responses, because hungry sheep would have had increased motivation for responding. Even so, the FR response rate of locoweed-treated sheep was significantly below baseline, and different from controls, even during transition weeks when locoweed was being reintroduced to the sheep.

Implications

The results indicate that in a 3-wk withdrawal period, sheep did not completely recover behaviorally after an initial 4-wk exposure to a low dose of the locoweed toxin. Additional exposure to locoweed reduced responses further, and poisoned sheep never completely recovered behaviorally. Locoweed-induced lesions in the brain apparently resolved within weeks of withdrawal of the toxin from the diet. This suggests that for "on-off" or cyclic grazing of locoweed-infested pastures to be successful, grazing periods must be kept very short with long withdrawal periods, particularly if sheep are avidly grazing the plant, so that irreversible damage will not result.

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